High level of genetic variation in *Aricia artaxerxes issekutzi* (Lycaenidae) populations in Northern Hungary

Katalin Pecsenye [1], Judit Bereczki [1], Mariann Szilágyi [1] & Zoltán Varga [1, 2]

[1] Department of Evolutionary Zoology and Human Biology, University of Debrecen, Debrecen, Egyetem tér 1., Hungary; e-mail: pecskati@tigris.unideb.hu
[2] HAS-DU Research Group for Evolutionary Genetics and Conservation Biology

Abstract. *Aricia artaxerxes issekutzi* imagos were collected from eight localities in two regions in Northern Hungary (Bükk Mountains and Aggtelek Karst region) in 1999 and 2000. Enzyme polymorphism was analysed at 16 enzyme loci using polyacrylamide gel electrophoresis. In the analysis of the data, F-statistics was computed and the total genetic variation was partitioned into within and between population components. Nei's genetic distances were calculated and UPGMA dendrogram was constructed on the basis of the distance matrix. Hierarchical F-statistics and AMOVA were computed to study the pattern of genetic differentiation among the samples. PCA analysis was also carried out using the allele frequencies of the samples. The Hungarian populations of *A. artaxerxes issekutzi* exhibited a high level of enzyme polymorphism. Both the dendrogram and the results of PCA indicated a clear differentiation between the Bükk and Karst regions. Most parameters of polymorphism and also the level of differentiation among the local populations were similar in the two regions. Nevertheless the average number of alleles per locus was significantly lower in the samples of the Bükk Mts than those of the Karst ones. In addition, the Bükk populations possessed a significantly lower portion of the species allele pool compared to the Karst ones. It was mostly the consequence of genetic drift due to the small population sizes in the Bükk Mts.

Introduction

Survival of natural populations in fragmented landscapes is an increasingly important topics in conservation biology (Ricketts 2001; Ries & Debinski 2001; Baguette & Schtickzelle 2001). Habitat fragmentation results in population subdivision and isolation. Consequently, it has grave influence on the genetic structure of populations resulting in decreasing effective population size and loss of genetic variation (Thomas et al. 1998). Nevertheless, different species may experience the same fragmented habitat in a different way (Thomas & Harrison 1992). Thus, surveys of genetic differentiation among local populations within a species have become more and more embedded in conservation studies (Schmitt & Hewitt 2004). Butterflies have been considered as sensitive indicators of changes in cultural landscapes. As a consequence, studies applying population genetic techniques to butterfly conservation surveys have assumed increasing importance.

Aricia artaxerxes (Fabricius, 1793) is a widespread Eurasiatic species. It occurs from Northern Europe to Central Asia and East Siberia (Obraztsov 1935, 1936). In the northwestern periphery of the range and also in the mountainous parts of southern Europe, it is subdivided into several subspecies (Beuret 1954; Kaaber & Hoegh-Guldberg 1961; Urbahn 1964; Hoegh-Guldberg 1966, 1968; Hoegh-Guldberg & Jarvis 1969; Kames 1969). One of these subspecies occurs in calcareous areas of the Carpathian basin such as the Bükk Mountains and the Aggtelek Karst region in Northern Hungary, described as *A. artaxerxes issekutzi* Balogh, 1956 (Varga 1961, 1968). The documented food plants of *A. artaxerxes issekutzi* are *Helianthemum ovatum* and *Geranium sanguineum*. However, the caterpillars of *A. artaxerxes* (different subspecies) have been

reared under artificial conditions on different *Geranium* species and *Erodium cicutarium* (Hoegh-Guldberg & Jarvis 1969; Varga pers. obs.). The favoured habitats of this subspecies are dry or semi-dry swards rich in flowers. *Aricia artaxerxes issekutzi* is a monovoltine species. Females lay their eggs usually singly on the lower surface of the leaves or on the flower buds of the food plant (Malicky 1969, Varga 1968). They have about 100 eggs but only 3–5 eggs will be layed on a plant. In this way, the eggs of a female are distributed fairly evenly within the habitat. Larvae develop first on the food plant. Later they are taken to ant nests. *Aricia artaxerxes*, however, is only a facultative myrmecophilous species (Malicky 1969).

The Brown Argus butterflies are known to be highly variable Lycaenid species (Aagard et al. 2002). They are, therefore, appropriate to analyze the genetic consequences of habitat degradation and fragmentation. The aim of the present study was to compare the level and structure of genetic variation in strong and declining populations of *A. artaxerxes issekutzi* in Northern Hungary.

Materials and Methods

Samples. *Aricia artaxerxes issekutzi* samples were collected from two subregions of northern Hungary: Aggteleki Karst region (strong populations) and Bükk Mountains (declining populations). Altogether 12 samples were collected from 8 populations in 1999 and 2000 (Fig. 1). In this way, the samples exhibited two types of hierarchy: geographic regions and populations within the regions (spatial pattern); years and populations collected in the same year (temporal pattern). Imagos were collected mostly in July, after the main egg-laying period and stored at –80°C until electrophoresis. Sample sizes varied between 12 and 40, according to the size of the populations.

Enzyme studies. Allozyme polymorphism was studied at 16 different loci by vertical polyacrylamide gel electrophoresis: aconitase (*Acon*), alcohol dehydrogenase (*Adh*), aldehyde oxidase (*Aox*), esterase (*Est*), glutamate dehydrogenase (*Gdh*), glutamate oxalacetate transaminase (*Got*), glucose-6-phosphate dehydrogenase (*G6pdh*), α-glycerophosphate dehydrogenase (*αGpdh*), hexokinase (*Hk*), isocitrate dehydrogenase (*Idh*), lactate dehydrogenase (*Ldh*), malate dehydrogenase (*Mdh*), malic enzyme (*Me*), phosphoglucose isomerase (*Pgi*), phosphoglucomutase (*Pgm*), and superoxid dismutase (*Sod*). Thoraxes were homogenized in 300μl extraction buffer and these samples were used to study *Got*, *αGpdh*, *G6pgdh*, *Hk*, *Idh*, *Ldh*, *Mdh*, *Me*, *Pgi*, *Pgm*, and *Sod*. Abdomens were homogenized in 150μl extraction buffer and these extracts were used to analyse *Acon*, *Adh*, *Aox*, *Est*, and *Gdh*. The extraction buffer, the electrophoresis buffer systems and running conditions, together with the staining solutions used for each enzyme are described in Bereczki et al. (2005). Genotypes of the different individuals were scored according to their enzyme pattern.

Statistical analyses. Genotype and allele frequencies were calculated on the basis of banding patterns. Measures of genetic variation (average number of alleles, % proportion of polymorphic loci, average observed heterozygosity) were calculated for each sample. The distribution of alleles with different frequencies between the two geographic regions was compared using generalised linear models. The computation was

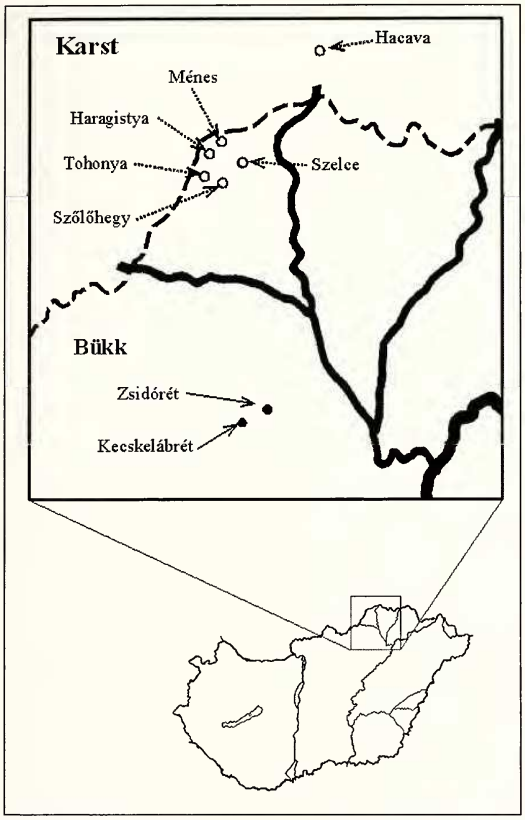


Fig. 1. Sample sites of *A. artaxerxes issekutzi* in northern Hungary.

carried out with GLIM 4 (Francis et al. 1994). Genetic differentiation among the populations was first analysed by Wright's F-statistics (Wright 1978; Weir 1996). In this analysis, the total genetic variation of the samples (F_{IT}) was partitioned into within (F_{IS}) and between population components (F_{ST}). The analyses were conducted by FSTAT version 1.2 (Goudet 1995). An exact test for population differentiation (Raymond & Rousset 1995a) was also conducted to test for independence of the allelic composition of the populations. Genepop, version 1.0 (Raymond & Rousset 1995b) was used to perform this test. Allele frequencies were used to estimate Nei's genetic distances (Nei 1972) and an UPGMA dendrogram (Sneath & Sokal 1973) was constructed on the basis of these data. The computation of genetic distances was performed by Biosys-1, Release 1.7 (Swofford & Selander 1981). The distribution of the total genetic variation at various levels of the hierarchy was also studied by AMOVA (Excoffier et al. 1992; Weir 1996). In this analysis, the total genetic variation is partitioned into three components: among groups, among populations within a group and within population. AMOVA was carried out by Arlequin, version 2.000 (Schneider et al. 2000). In the last part of the study, we carried out a principal component analysis (PCA) using the genotypic composition of the individuals to show the size of overlap in the genetic variation of the populations in a reduced space of variables. PCA analyses were performed running R Package Version 4.0 (Casgrain & Legendre 2001).

Results

Level of enzyme polymorphism. Three of the 16 loci analysed (*Hk*, *αGpdh*, *Sod*) did not have an alternative allele in any of the investigated sample. The samples exhibited a very high level of polymorphism. As a whole, the portion of polymorphic loci was 57.3% and the average frequency of heterozygotes was 24.6% (Tab. 1). Heterozygote deficiency was observed in all samples, which proved to be significant in 8 cases out of the total 12 (Tab. 1). The total number of alleles was 71 at the 16 loci investigated. We have calculated the portion of alleles the samples actually possessed of the total 71 (Tab. 1: A). In general, populations contained 64% of the species gene pool. The

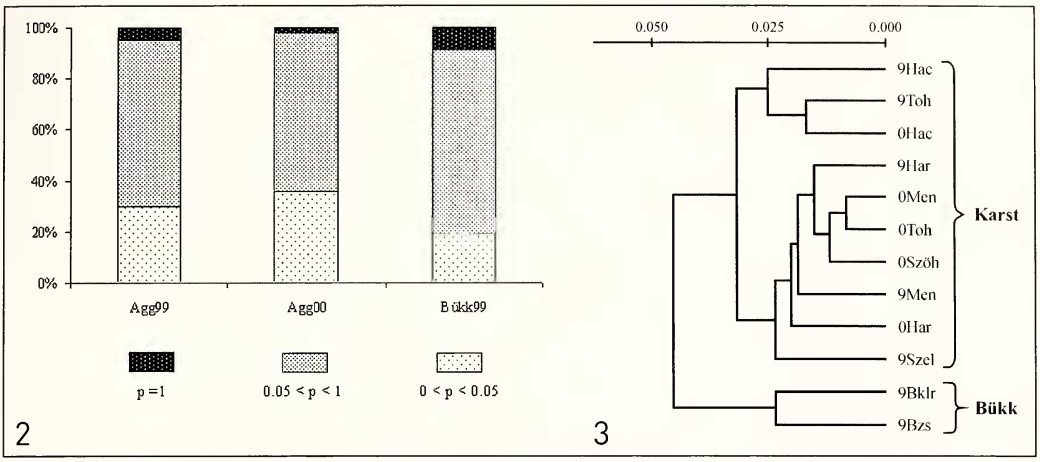
Tab. 1. Parameters of enzyme polymorphism in the *Aricia artaxerxes issekutzi* samples. **N**: sample size; $\mathbf{n_A}$: average number of alleles per locus; A: average portion of the allele pool of the species; **P**: portion of the polymorphic loci; $\mathbf{F_{IS}}$: index representing the within population component of genetic variation. *: significant at 0.05 level; **: significant at 0.01 level; ***: significant at 0.001 level.

		N	n_A	A	P	H	F_{IS}
Bükk 99	Kecskelr.	22.5	2.19	49.3	56.3	0.214	0,139**
	Zsidórét	15.8	2.63	59.2	56.3	0.265	0.043
	Subtotal	**19.2**	**2.41**	**54.3**	**56.3**	**0.240**	**0.098***
Karst 99	Hacava	23.8	3.13	70.4	68.8	0.274	0.185***
	Haragistya	31.5	2.75	62.0	50.0	0.227	0.137**
	Ménes	26.6	3.13	70.4	56.3	0.244	0.147**
	Szelce	12.0	2.44	54.9	50.0	0.234	0.071
	Tohonya	14.8	2.63	59.2	56.3	0.250	0.107
	Subtotal	**21.7**	**2.82**	**63.4**	**56.28**	**0.246**	**0.141****
Karst 00	Hacava	32.5	3.31	77.5	68.8	0.258	0.122**
	Haragistya	35.1	3.06	69.0	56.3	0.249	0.029
	Ménes	21.8	2.88	64.8	50.0	0.239	0.132*
	Szőlőh.	25.8	2.88	69.0	56.3	0.255	0.093*
	Tohonya	39.4	2.88	64.8	62.5	0.242	0.137**
	Subtotal	**30.9**	**3.00**	**69.0**	**58.8**	**0.249**	**0.103****
Total			**2.66**	**64.2**	**57.3**	**0.246**	**0.117****

Tab. 2. Results of F-statistics for the *A. artaxerxes issekutzi* samples. F_{IT}: index indicating the total genetic variation of the samples; F_{IS}: index representing the within population component; F_{ST}: fixation index. *: significant at 0.05 level; **: significant at 0.01 level.

Loci	F_{IT}	F_{IS}	F_{ST}
Acon	0.210**	0.154**	0.066**
Adh	0,211**	0.202**	0.011*
Aox	0.173**	0.127*	0.053**
Est	0,226**	0.205**	0.026**
Gdh	0.232**	0.217*	0.019
Got	0.072	-0.010	0.081**
G6pdh	-0.005	-0.004	-0.001
Idh	-0.010	-0.011	0.001
Ldh	0.288**	0.289**	-0.001
Mdh	0.014	-0.005	0.019
Me	0.216**	0.191**	0.032**
Pgi	0.033	0.026	0.007*
Pgm	0.078*	0,060	0.019**
Total	0.146**	0.117**	0.033**

samples were grouped in two ways. First, the samples collected in 1999 were split according to their geographic origin (Bükk vs. Karst populations). Except for the parameters indicating allelic richness, there was no significant difference in the level of polymorphisms between the samples collected in the two geographic regions. Both the average number of alleles per locus ($F_{1,10}$=8.09; 0.05>P>0.01) and the portion of the species gene pool the population possessed (χ^2_1=7.73 0.01>P>0.001) were, however, significantly higher in the populations collected in the Karst region compared to those of the Bükk Mts. The next step was to sort the samples of the Karst region according to the years (generations) they were collected. Although the samples collected in 2000 exhibited a slightly higher polymorphism these differences were not significant for any of the parameters (Tab. 1). We also compared the distribution of alleles with different frequencies between



Figs 2–3. 2. Distribution of alleles with different frequencies in the populations of the two regions in the two years of collection. Agg99: Karst samples collected in 1999; Agg00: Karst samples collected in 2000; Bükk99: samples collected in the Bükk plateau in 1999. **3.** UPGMA dendrogram of the *A. artaxerxes issekutzi* samples constructed on the basis of Nei's distances.

the two regions and between the two years. Three frequency categories were set: rare alleles ($p<0.05$), common alleles with a frequency of $0.05<p<1$ and fixed alleles ($p=1$). The differences between the two regions in their allele distribution were significant ($\chi^2_2=8.04$; $0.05>P>0.01$). The Bükk populations carried less rare alleles but were fixed for more alleles than the Karst populations (Fig. 2). At the same time, the two samples collected in the Karst region proved to be similar ($\chi^2_2=4.04$; $P>0.05$).

Structure of the genetic variation. The results of F-statistics indicated that a substantial portion of this variation was observed within the samples. The average F_{IS} value suggested significant heterozygote deficiency within the samples (Tab. 2), which was attributable to 7 of the 13 loci. At the same time, the samples exhibited a relatively low level of genetic differentiation (Tab. 2: F_{ST}). Yet, 8 of the total 13 loci proved to be significantly differentiating (Tab. 2).

Nei's (1972) genetic distances were calculated and a UPGMA dendrogram was constructed on the basis of the distance matrix. The dendrogram showed an obvious geographic pattern (Fig. 3). The samples originating from the two regions were clustered in two well differentiated branches. Moreover, the two samples collected in Hacava, which is situated in the Slovakian part of the Karst region were separated from the other Karst samples. At the same time, the samples of the other Karst populations (except for the '99 sample from the Tohonya ridge) collected in the two consecutive years (generations) were scattered randomly in the middle branch (Fig. 3). This indicates a fairly high level of random changes in their allele frequencies.

In the analysis of the structure of genetic differentiation, we contrasted the spatial and temporal components of the between sample variation. Accordingly, we computed AMOVA using Arlequin. In this analysis, the total genetic variation can be analysed involving the within sample component as well. However, we could not include all levels of the hierarchy above the sample in a single run. We, therefore, conducted the analy-

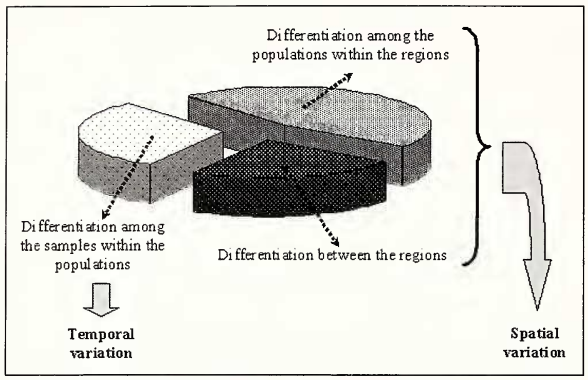


Fig. 4. Distribution of the total between sample variation in *A. artaxerxes issekutzi*.

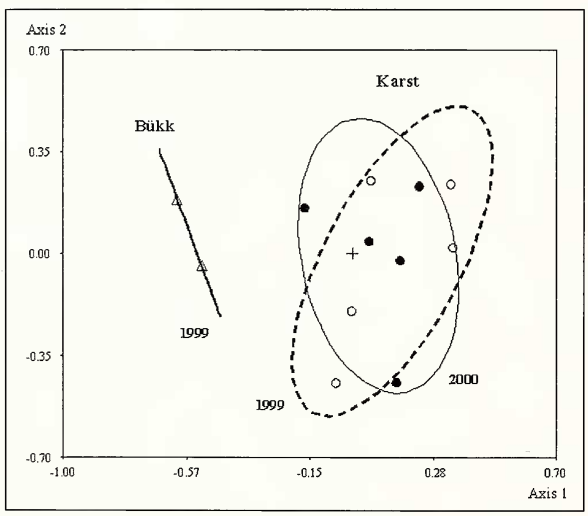


Fig. 5. Results of the PCA analysis for the *A. artaxerxes issekutzi* samples. △: Bükk samples; ●: Karst samples collected in 1999; ○: Karst samples collected in 2000.

Region	Year	F_{ST}	Exact test
Karst	1999	0.024** (6)	*** (3)
	2000	0.020** (6)	*** (6)
	Total	0.024** (6)	*** (6)
Bükk	1999	0.022* (3)	* (2)
Total		0.033** (8)	*** (7)

ses in two series. In the first run, the levels of the hierarchy were: regions (group of samples) and samples. In the second run, the following levels of the hierarchy were considered: populations (group of samples) and samples. The results suggested that most of the total genetic variation was attributable to the within sample component (95.08%). The distribution of the between sample variation revealed that a much higher portion of variation was explained by the differences among the populations (Fig. 4: spatial variation) than by those within them, i.e. between the samples collected in different generations from the same population (Fig. 4: temporal variation). The indices of differentiation also indicated that the spatial component of variation was far larger than the temporal one. F_{PT}, the index representing the level of differentiation among the populations was highly significant (F_{PT}= 0.034, P<0.001) while F_{SP}, the index representing the level of differentiation among the samples within the populations was not (F_{SP}= 0.011, P>0.05).

Similarly to the dendrogram, the results of the PCA analysis also indicated a clear geographic pattern of differentiation. The samples exhibited two large clouds of points

Tab. 3. F_{ST} values and the results of exact test at different levels of the hierarhy in the *A. artaxerxes issekutzi* samples. The numbers in brackets indicate the significantly differentiating loci. *: significant at 0.05 level; **: significant at 0.01 level; ***: significant at 0.001 level.

Tab. 4. Results of AMOVA for the *A. artaxerxes issekutzi* samples. **BR**: between region variation; **WRBP**: between population variation within a region; **WPBS**: between sample (year/generation) variation within a population; **WS**: within sample (year/generation) variation. Indices of differentiation were determined in two analyses with different levels of hierarchy: **Analysis 1** – region and sample; **Analysis 2** – population and sample. $\mathbf{F_{RT}}$: index of differentiation among the regions; $\mathbf{F_{PT}}$: index of differentiation among the populations; $\mathbf{F_{SR}}$: index of differentiation among the samples (year/generation) within a region; $\mathbf{F_{SP}}$: index of differentiation among the samples (year/generation) within a population; $\mathbf{F_{ST}}$: index of total differentiation among the samples (year/generation).

% of variation		Indices of differentiation		
			Analysis 1	Analysis 2
BR	1.14	F_{RT}	0.034***	-
WRBP	2.29	F_{PT}	-	0.024***
WPBS	1.49	F_{SR}	0.026***	-
WS	95.08	F_{SP}	-	0.011
Total	100	F_{ST}	0.059***	0.035***

in the reduced space of variables (Fig. 5). The two axes explained 56.7% of the genetic variation of the samples. The 95% ellipses drawn according to the geographic regions and the years the samples were collected indicated an evident separation between the Karst and Bükk populations along the first axis. The allele frequency distribution at the *Got* and *Aox* loci contributed most to this axis. In accordance with the results of the hierarchcal F-statistics the samples collected in two consecutive years/generations in the Karst populations comprised largely overlapping clouds.

Discussion

In line with other European lycaenid butterflies studied we found a high level of polymorphism in *Aricia artaxerxes issekutzi* populations. *Polyommatus coridon*, *P. bellargus* and *P. icarus* has been surveyed in many Western and Central European populations (Schmitt & Seitz 2001a, b; Schmitt et al. 2003). Those results revealed a high level of polymorphism e.g. the proportion of polymorphic loci ranged from 0.42 to 0.85. The mean number of alleles per locus has been especially high (3.0-3.5) in the southern European *P. coridon* populations (Schmitt & Seitz 2001a; Schmitt et al. 2002). Aagaard et al. (2002) have reported a high level of genetic variation in the northern European populations of *Aricia artaxerxes* and *A. agestis*. They found 1.84 alleles per locus on average with some loci having 6 or even 8 alleles. Our data indicated an even higher polymorphism than those of Aagaard et al. (2002). In the Hungarian *Aricia* populations, the average number of alleles ranges between 2.2 and 3.3. Moreover, we detected 17, 11 and 9 alleles at the *Est*, *Pgi* and *Pgm* loci respectively. As a consequence of the high number of alleles per locus, we also observed an exceptionally high frequency of heterozygotes (average H_o=0.246). As far as known, in butterfly populations enzyme studies has only revealed such a high level of heterozygosity in *P. hispana* populations (Schmitt et al. 2005).

Although most parameters of polymorphism were similar in the two regions studied, the average number of alleles per locus and the average portion of the species gene

pool each population possessed were significantly lower in the Bükk samples than in the Karst ones. Moreover, the distribution of alleles among the three frequency categories (rare, common and fixed alleles) was also different in the two regions. Namely, the fixed alleles were more frequent in the Bükk populations, whereas the rare alleles were more common in the Karst ones. It thus appears that populations living in the Bükk plateau have a lower allelic richness than those of the Karst region. A possible explanation of this situation is that *Aricia* populations are smaller and more isolated in the Bükk plateu than in the Karst region. *Aricia artaxerxes* prefers short-grass habitats at moderately high altitudes, which are rich in flowering dicotyledonous plants. The main nectar sources of this species are small Fabaceae species with yellow flowers like *Lotus corniculatus*, *Melilotus spp.* (Varga pers. obs.). Moreover, females should find the small larval food plants in the lower vegetation.

These circumstances can only be maintained under suitable edaphic-microclimatic conditions, e.g. at the rupicolous margin of karstic dolinas and/or by appropriate management. In general, mountain grasslands, however, are prone to succession process without appropriate management. The structure will change due to overgrowth by tall grasses and by the extension of shrubby vegetation. This process has been quite rapid on the higher (about 800–850 m) and more humid (over 800 mm precipitation pro year) Bükk plateau as a consequence of abandoned mowing. Accordingly, *Aricia artaxerxes issekutzi*, which was fairly common in the sixties (Varga pers. obs.) became rare and more localised during the last two decades. Management has started in the last 2–3 years in order to save these populations.

The situation is quite different in the Aggtelek Karst region. Lower precipitation (about 600 mm per year) and the shallow, karstic substrate is associated with an essentially slower succession. As a consequence, a more favourable structure and diversity of vegetation has been maintained in the Karst region than on the Bükk plateau. Both short-grass swards with abundant *Helianthemum ovatum* and xerothermic forest-steppe fringes with *Geranium sanguineum* remained widely distributed. Moreover, these habitat patches compose a kind of network on the Karst plateaus facilitating migration. Thus, *Aricia* populations have remained rather strong there. In contrast, the suitable habitat patches have bocome much smaller and relatively isolated in the Bükk Mts. The consequence of small population size and isolation is the enhanced effect of genetic drift, which results in an increased probability of allele fixation and loss of rare alleles (Frankham et al. 2002; Allendorf & Luikart 2006 and references therein). We detected both of these symptoms in the Bükk populations.

The level of differentiation was significant among the Hungarian *A. artaxerxes issekutzi* samples. Aagard et al. (2002) also detected significant inhomogeneity among the *A. artaxerxes* populations in the UK and Scandinavia. Assuming the relatively strong effect of genetic drift in the Bükk populations we expected a higher level of differentiation in this region than in the Karst one. Contrary to our presumption, the F_{ST} values indicated a fairly similar level of genetic differentiation in the two regions. This suggests that the size of the Bükk populations has decreased recently, i.e. they have been exposed to the effect of genetic drift for a short period. Though they have lost several rare alleles and a number of loci have become fixed this time period has not been long enough to enhance genetic differentiation among the local populations.

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References

Aagaard, K., K. Hindar, A. S. Pullin, C. H. James, O. Hammarstedt, T. Balstad & O. Hanssen 2002. Phylogenetic relationship in brown argus butterflies (Lepidoptera: Lycaenidae: Aricia) from north-western Europe. – Biological Journal of the Linnean Society **75**: 27–37.

Allendorf, F. W. & G. Luikart 2006. Conservation and the Genetics of Populations. – Blackwell Publishing, Malden, USA.

Baguette, M. & N. Schtickzelle 2001. Local population dynamics are important to the conservation of metapopulations in highly fragmented landscapes. – Journal of Animal Ecology **40**: 404–412.

Bereczki, J., K. Pecsenye, L. Peregovits & Z. Varga 2005. Pattern of genetic differentiation in the *Maculinea alcon* species group (Lepidoptera, Lycaenidae) in Central Europe. – Journal of Systematics and Evolutionary Research **43**: 157–176.

Beuret, H. 1954. Die Lycaeniden der Schweiz. Teil II. – Mitteilungen der Entomologische Gesellschaft Basel (N.F.) 4, suppl.: 204–222.

Casgrain, P. & P. Legendre 2001. The R package for multivariate and spatial analysis, version 4.0 d5 User's manual. <http://www.fas.unmontreal.ca/BIOL/legendre/>

Excoffier, L., P. Smouse & J. Quattro 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. – Genetics **131**: 479–491.

Francis, B., M. Green & C. Payne 1994. GLIM 4. The statistical system for generalised linear interactive modelling. – New York University Press Inc.

Frankham, R., J. D. Ballou & D. A. Briscoe 2002. Introduction to Conservation Genetics. – Cambridge University Press, Cambridge.

Goudet, J. 1995. Fstat version 1.2: a computer program to calculate F-statistics. – Journal of Heredity **86**: 485.

Hoegh-Guldberg, O. 1966. North European groups of *Aricia allous* G-Hb. Their variability and Relationship to *A. agestis* Schiff. – Natura Jutlandica **13**: 1–184.

Hoegh-Guldberg, O. 1968. Evolutionary trends in the genus Aricia. – Natura Jutlandica **14**: 1–76.

Hoegh-Guldberg, O. & F. V. L. Jarvis 1969. Central and North European Ariciae (Lep.). Relationships, Heredity, Evolution. – Natura Jutlandica **15**: 1–106.

Kaaber, S. & O. Hoegh-Guldberg 1961. Aricia allous Hb. ssp. vandalica nov. – Flora og Fauna. Meddelse fra Naturhistorisk Museum Aarhus **67**: 122–128.

Kames, P. 1969. Aricia artaxerxes spp. nova hercynica. – Natura Jutlandica **15**: 107–119.

Malicky, H. 1969. Übersicht über Präimaginalstadien, Bionomie und Ökologie der mitteleuropäischen Lycaenidae (Lepidoptera). – Mitteilungen der entomologischen Gesellschaft Basel **19**: 25–91.

Nei, M. 1972. Genetic distance between populations. – The American Naturalist **106**: 283–292.

Obraztsov, S. 1935. Lycaena (Aricia) allous Hb. und ihre östlichen Rassen. – Folia Zoologica et Hydrobiologica Riga **8**: 141–150.

Obraztsov, S. 1936. Weiteres über Lycaena (Aricia) allous Hb. – Folia Zoologica et Hydrobiologica Riga **9**: 58–61.

Raymond, M. & F. Rousset 1995a. An exact test for population differentation. – Evolution **49**: 1280–1283.

Raymond, M. & F. Rousset 1995b. GENEPOP ver.1.2, a population genetics software for exact tests and ecumenicism. – Journal of Heredity **86**: 246–249.

Ricketts, T. H. 2001. The Matrix Matters: Effective Isolation in Fragmented Landscapes. – The American Naturalist **158**: 88–99.

Ries, L. & D. M. Debinski 2001. Butterfly responses in habitat edges in the highly fragmented prairies of Central Iowa. – Journal of Animal Ecology **70**: 840–852.

Schmitt, Th. & G. M. Hewitt 2004. The genetic pattern of populationthreat and loss: a case study of butterflies. – Molecular Ecology 13: 21-31.

Schmitt, Th. & A. Seitz 2001a. Allozyme variation in *Polyommatus coridon* (Lepidoptera, Lycaenidae): identification of ice-age refugia and reconstruction of postglacial expansion. – Journal of Biogeography **28**: 1129–1136.

Schmitt, Th. & A. Seitz 2001b. Intraspecific allozymatic differentiation reveals the glacial refugia and the postglacial expansions of European *Erebia medusa* (Lepidoptera: Nymphalidae). – Biological Journal of the Linnean Society **74**: 429–458.

Schmitt, Th., A. Giessl & A. Seitz 2002. Postglacial colonisation of western Central Europe by *Polyommatus coridon* (Poda, 1761) (Lepidoptera, Lycaenidae): evidence from population genetics. – Heredity **88**: 26–34.

Schmitt, Th., A. Giessl & A. Seitz 2003. Did *Polyommatus icarus* (Lepidoptera, Lycaenidae) have distinct glacial refugia in southern Europe? Evidence from population genetics. – Biological Journal of the Linnean Society **80**: 524–538.

Schneider, S., D. Roessli & L. Excoffier 2000. Arlequin ver. 2.000: A software for population genetic data analysis. – Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sneath, P. H. & R. R. Sokal 1973. Numerical Taxonomy. – W. H. Freeman, San Francisco.

Swofford, D. L. & R. B. Selander 1981. Biosys-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. – Journal of Heredity **72**: 281–283.

Thomas, C. D. & S. Harrison 1992. Spatial dynamics of a patchily distributed butterfly species. – Journal of Animal Ecology **61**: 437–446.

Thomas, C. D., J. K. Hill & O. T. Lewis 1998. Evolutionary consequences of habitat fragmentation in a localised butterfly. – Journal of Animal Ecology **67**: 485–497.

Urbahn, E. 1964. Neuere Auffassungen in der *Maculinea alcon-rebeli*-Frage (Lepidoptera, Lycaenidae). – Entomologische Abhandlungen, Staatliches Museum für Tierkunde, Dresden **32**: 69–77.

Varga, Z. 1961. Zoogeographical survey of the Macrolepidoptera-fauna of the N-Hungarian Karst area (in Hung. with German summary). – Folia entomologica hungarica **14**: 345–386.

Varga, Z. 1968. Bemerkungen und Ergänzungen zur taxonomischen Beurteilung und Ökologie der im Karpatenbecken vorkommenden Populationen von *Aricia artaxerxes* Fabr. (= A. allous G.-Hb., A. montensis Vrty., Lepidoptera, Lycaenidae) – Acta Biologica Debrecina **6**: 171–185.

Weir, B. S. 1996. Genetic Data Analysis II: Methods for Discrete Population Genetic Data. – Sunderland, Sinauer Associates, Inc.

Wright, S. 1978. Evolution and the Genetics of Populations. vol. 4. Variability Within and Among Natural Populatons. – University of Chicago Press, Chicago.